AD NUMBER

ADB266141

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; Oct 2000. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, MD 21702-5012

AUTHORITY

USAMRMC ltr, 8 Jan 2003

AD_____

Award Number: DAMD17-98-1-8106

TITLE: BRCA1 and BRCA2 Mutations in African Americans

PRINCIPAL INVESTIGATOR: Carolyn W. Broome, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Washington, DC 20059

REPORT DATE: October 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

NOTICE

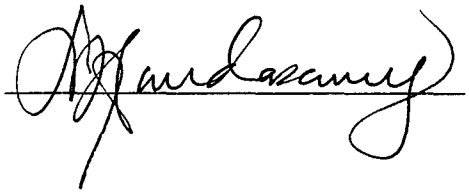
USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-98-1-8106
Organization: Howard University
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.



04/26/07

_____ _____

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2000	Annual (14 Sep 99 - 13 Sep 00)

4. TITLE AND SUBTITLE
BRCA1 and BRCA2 Mutations in African Americans

5. FUNDING NUMBERS
DAMD17-98-1-8106

6. AUTHOR(S)
Carolyn W. Broome, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Howard University
Washington, DC 20059

E-MAIL:
cbroome@fac.howard.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Oct 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns are being examined in breast cancer patients from 80 families at high-risk of hereditary breast cancer. This study represents one of the largest studies of high-risk African Americans. One deleterious *BRCA1*mutation and eight pathogenic *BRCA2* mutations have been identified. The *BRCA1* mutation and 38% of the *BRCA2* mutations are unique to African Americans. Two *BRCA2* mutations were observed in male breast cancer patients. All of the female breast or ovarian cancer probands with *BRCA2* mutations were diagnosed before the age of 45.

The importance of this work is for genetic testing and counseling. Sixty-four percent of African American *BRCA1* and *BRCA2* cases have at least one affected first degree relative. It is noteworthy that 36% of *BRCA* mutations were detected in individuals without a reported history of disease, among early onset (≤40 years) breast cancer patients, male breast cancer patients, and a patient with both breast and ovarian cancers. Because of the numerous, distinct pathogenic *BRCA* mutations in African Americans, genetic testing for *BRCA* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, African American, *BRCA1, BRCA2*, mutation detection	32
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

TABLE OF CONTENTS

INTRODUCTION

BRCA1 and *BRCA2*. Specific breast cancer predisposing mutations in *BRCA1* and *BRCA2* have been associated with different ethnic groups; therefore mutations in African Americans are expected to differ from those reported in other populations. Most studies of hereditary breast cancer have included few African Americans (Miki et al., 1994; Futreal et al. 1994; Castilla et al., 1994). In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns are being examined in 80 breast cancer patients from families at high-risk of hereditary breast cancer. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations. Mutations/variations detected in high-risk families are also being tested in controls, unselected for disease. This investigation will provide information for breast cancer genetic testing and genetic counseling in African Americans.

BODY

Task 4: To collect more blood samples from high-risk African American breast cancer patients, low-risk patients and controls. High-risk criteria are multiple cases (including first-degree, second-degree, and distant relatives in the same lineage) of breast cancer or multiple cases of breast and ovarian cancer per family; or breast cancer with early age of onset ($\leq$ 40 years); or bilateral breast cancer; or breast and ovarian cancer in the same individual; or male breast cancer (**Table 1**, appendix). During the first 24 months, we have recruited 105 additional subjects: 34 affected and unaffected members of high risk families; 3 low-risk breast cancer patients, who do not meet the high-risk criteria; 68 control subjects, who do not have breast cancer or belong to families with multiple cases of breast cancer. Therefore, the total number of high-risk families is 80; the total number of control subjects is 163. There are 87 low-risk families.

Task 1: To identify *BRCA1* mutations in additional high-risk African American families. *BRCA1* mutation analysis using single-stranded conformational polymorphism (SSCP) followed by sequencing of variants in high-risk African American families has been extended from 45 families **(Panguluri et al., 1999; Mefford et al., 1999)** to 55 families as proposed in Task 1. Of the 10 additional families, one pathogenic mutation, 2495insG, was detected in a family with 4 cases of breast cancer (**Table 2**). This frameshift mutation is expected to produce a protein termination at amino acid residue 800, presumably deleting 57% of the BRCA1 protein. Several polymorphisms and a variation in intron 7 were also detected; these are probably not disease associated.

Task 2: To identify *BRCA2* mutations in high-risk African American families. *BRCA2* mutation analysis of the entire coding sequence and flanking introns has been completed in 74 high-risk African American families, using the protein truncation test (PTT) and SSCP followed by sequencing of variants **(Whitfield-Broome et al., 1999; Whitfield-Broome et al., 2000; Kanaan et al., 2000a-e**; **Olopade et al., 2000**, see Appendix**).** Presentations have been made at numerous scientific meetings; see section on Reportable Outcomes.

Eight protein truncating, pathogenic mutations have been detected **(Table 3)**. Three (1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described. Therefore, about 38% of the pathogenic

mutations observed are unique to African Americans. The other five pathogenic mutations (1882delT, 1991delATAA, 2816insA, 4075delGT, 4088delA) detected in African Americans have been reported in Caucasians. One of these protein truncating mutations, 2816insA, has been reported in both African American and Caucasian populations (Gao et al., 2000; BIC). Two of the pathogenic mutations, 1991delATAA and 2816insA, were detected in male breast cancer patients, consistent with previous studies showing an association of *BRCA2* with male breast cancer (Ford et al., 1998). One-half of the pathogenic mutations were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease. All breast or ovarian female probands with *BRCA2* mutations were diagnosed before the age of 45.

A novel missense variant H2395L of unknown functional significance was observed **(Table 4)**. This variant is currently being tested for segregation with disease in this family. Numerous silent amino acid changes, noncoding variations, and polymorphisms, which may not be disease associated, were also observed **(Tables 4, 5)**. None of the intron variations are expected to affect splicing. The deletion of T at IVS12 + 3 shifts a conserved nucleotide (A) into that position. Many of these variants have been reported previously to occur globally in control populations (Wagner et al., 1999; BIC).

Task 4: To screen control subjects for *BRCA* mutations detected in high-risk African American patients. Neither the protein truncating mutations, nor rare missense, rare silent, rare non-coding variations have been detected in 163 controls. The polymorphisms are currently being tested in the control samples.

Task 3: To functionally test *BRCA1* and *BRCA2* missense or intron mutations. This will be undertaken if a missense or intron mutation segregates with disease in the family and the wild type amino acid or intron nucleotide is conserved in other mammalian species.

Discussion. The entire coding region and flanking introns of *BRCA1* and *BRCA2* have been examined in 51 high-risk breast cancer patients. Three female and 2 male pathogenic *BRCA2* mutations and 3 female pathogenic *BRCA1* mutations have been detected. Usually the number of *BRCA1* mutations exceeds the number of *BRCA2* mutations (Ford et al., 1998). Our observation or more *BRCA2* than *BRCA1* mutations could be a function of our high-risk population: 59% is over the age of 40; 3% is male breast cancer; and only 4% represents breast and ovarian families. *BRCA1* mutations account for the majority of mutations in breast and ovarian families; whereas, *BRCA2* mutations occur more frequently in older patients and in male patients (Krainer et al., 1997; Ford et al., 1998).

Among multiple case African American families with only breast cancer (8%, our study; 13%, Gao et al.,2000), the frequency of *BRCA2* mutations is about the same as in Caucasian families (11%, Frank et al., 1998). The percentage of *BRCA2* mutations among families with breast and ovarian cancers appears to be higher in African Americans (40%, our study; 33%, Gao et al., 2000) than in Caucasians (15%, Frank et al., 1998); however, this may be a function of the small number of African American cases. The percentage of *BRCA2* mutations among African American families with 6 or more breast cancer cases (40%, 2/5) is the same as in comparable Caucasian families (44%, Ford et al., 1998).

Of the *BRCA2* mutations, 62.5% (5/8) were detected in families with less than 3 affected cases of breast or ovarian cancer. Among the *BRCA2* carriers, 37.5% (3/8, two females

diagnosed at < 40 years of age and one male) reported no family history of breast or ovarian cancer.

Considering all of our African American *BRCA1* and *BRCA2* cases, 64% (7/11) have at least one affected first degree relative. Nevertheless, a significant minority (36%, 4/11, including 2 early onset females, 1 male, and 1 female with breast and ovarian cancers) of our *BRCA1* and *BRCA2* carriers report no family history of breast or ovarian cancers. This may be due to a reluctance of African American family members to discuss their diseases. Therefore, the guidelines for family history used for Caucasians in recommending genetic testing may miss a significant percentage of African American *BRCA* carriers.

Our original hypothesis was that by studying a large number of high-risk African Americans with a family history of breast/ovarian cancer or early-onset breast cancer, we would detect the *BRCA1* and *BRCA2* mutations that are most common in the African American population. We expected that mutations in African Americans would differ from those reported in other populations. Our data support this hypothesis. Thirty-eight percent (3/8) of our *BRCA2* mutations are unique to the African American population.

In my analysis of our results and those of others, fifty-nine percent (16/27) of pathogenic *BRCA1* mutations (protein truncating, disease-associated missense, splicing) and 50% (9/18) of *BRCA2* mutations are unique to patients of African ancestry (Olopade et al., 2000). Only 26% (7/27) of the pathogenic *BRCA1* and 17% (3/18) of the pathogenic *BRCA2* mutations have been identified in more than one family. Two deleterious *BRCA1* mutations (7%) and no deleterious *BRCA2* mutations have been observed in more than 2 African American families. Therefore, African Americans exhibit a unique broad spectrum of *BRCA1* and *BRCA2* mutations.

One purpose of this research was to provide information for genetic testing and counseling. The numerous, distinct pathogenic mutations in *BRCA1* and *BRCA2* observed in African Americans reflects the high level of genetic diversity in people of African ancestry (Jorde et al., 1998). Because of this broad spectrum of distinct mutations, genetic testing for *BRCA1* and *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

KEY RESEARCH ACCOMPLISHMENTS

- Additional high-risk families have been recruited; there are now 80 high-risk families.
- A novel *BRCA1* pathogenic, protein truncating mutation has been identified in a multiple case family.
- Eight pathogenic, protein truncating *BRCA2* mutations have been identified among 74 high-risk African American patients, three of which are unique to African Americans.
- A *BRCA2* missense variant of unknown functional significance was detected. Numerous polymorphisms and noncoding variants, which have been detected globally, were observed in the *BRCA1* and *BRCA2* genes.
- Neither the protein truncating mutations, nor rare missense, rare silent, rare non-coding variations have been detected in 163 controls.
- The distribution and frequency of African American *BRCA1* and *BRCA2* mutations have been analyzed in terms of family history and ovarian cancers and compared to Caucasians.

- The spectrum of pathogenic *BRCA1* and *BRCA2* mutations in African Americans from this study and others has been analyzed.

REPORTABLE OUTCOMES

Abstracts and Presentations (*presenter)

Whitfield-Broome, C.*, Kanaan, Y., Kpenu, E., Utley, K., Dunston, G.M., Brody, L.C. (2000) BRCA2 mutations in African Americans. Poster presentation, Era of Hope Department of Defense Breast Cancer Research Program Meeting, Atlanta, GA., June, 2000.

***Whitfield-Broome C**, (1999) Inheritance of Breast Cancer in African American Women: How Should We Monitor? Presentation, Howard University Women's Health Institute, Washington DC, April, 1999. The Proceedings of Health Issues and Concerns of Women of Color: A Call to Action, Health and Human Services, in press.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000a) BRCA2 mutations in African Americans. Poster presentation, American Society of Human Genetics, Philadelphia, October, 2000. Am. J. Hum. Genet. (supplement 2) 67:92 abs. #455

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000b) BRCA2 mutations in African Americans. Poster presentation, Howard University, Graduate School of Arts and Sciences, April, 2000. First place award to Y.K.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000c) BRCA2 mutations in African Americans. Oral & poster presentation, Howard University Medical Center Scientific Forum, May, 2000. Second place Roland & Waldean Nickens scientific research award to Y.K.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000d) BRCA2 mutations in African Americans. Oral & poster presentation, American Society for Microbiology, Washington D.C. branch, student meeting, May, 2000 Third place award to Y.K.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000e) BRCA2 mutations in African Americans. Oral & poster presentation, National Medical Association, August, 2000. Third place award to Y.K.

Olopade, O.*, Dunston, G., Tainsky, M., Collins, F., **Whitfield-Broome. C.** (2000) Breast cancer genetics. Invited presentation, Summit meeting evaluating research on breast cancer in African American women, Washington D.C., September, 2000. Manuscript in preparation.

Publications on *BRCA1/2* (work performed before this grant)

Whitfield-Broome, C., Dunston, G.M., Brody, L.C. (1999) BRCA2 Mutations in African Americans. American Association for Cancer Research, Philadelphia, April, 1999. Proc. Amer. Assoc. for Cancer Research, 40:269, abstract #1788

Panguluri RCK, Brody LC, Modali R, Utley K, Adams-Campbell L, Day AA, **Whitfield-Broome C,** & Dunston GM. (1999) BRCA1 mutations in African Americans Human Genetics 105:28-31 and on line http://dx.doi.org/10.1007/s004399900085.

Mefford HC, Baumbach L, Panguluri RCK, **Whitfield-Broome C**, Szabo C, Smith S, King MC, Dunston G, Stoppa-Lyonnet D, Arena F. (1999) Evidence for a BRCA1 founder mutation in families of west African ancestry. Am J Hum Genet 65:575-578.

Training

As provided in the approved budget, a Ph.D. graduate research assistant, Yasmine Kanaan is working on this project and has presented her work at local and national meetings, receiving several awards for her presentations (see above).

CONCLUSIONS

In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns have been examined in breast cancer patients from families at high-risk of hereditary breast cancer. Additional high-risk families have been recruited so that there are now 80 high-risk families. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations. Presently, 51 families have been screened for both *BRCA1* and *BRCA2* mutations; 74 families have been examined for *BRCA2* mutations

- A novel *BRCA1* pathogenic, protein truncating mutation has been detected in a multiple case family.
- Eight pathogenic *BRCA2* mutations have been identified; three of which (38%) are unique to the African American population. Two *BRCA2* mutations were observed in male breast cancer patients. None of the pathogenic *BRCA2* mutations were carried by 163 controls.
- All of the female breast or ovarian cancer probands with *BRCA2* mutations were diagnosed before the age of 45.
- A *BRCA2* missense variant of unknown functional significance was detected. Numerous polymorphisms and noncoding variants, which have been detected globally, were observed in the *BRCA1* and *BRCA2* genes.
- Among multiple case African American families with breast cancer only, the frequency of *BRCA2* mutations (8%) is about the same as in Caucasian families.
- The percentage of *BRCA2* mutations among families with breast and ovarian cancers may be higher in African Americans (40%) than in Caucasians (15%).
- The percentage of *BRCA2* mutations among African American families with 6 or more breast cancer cases (40%) is the same as in comparable Caucasian families.
- Considering all of our African American *BRCA1* and *BRCA2* cases, 64% (7/11) have at least one affected first degree relative.
- Nevertheless, a significant minority (36%) of our *BRCA1* and *BRCA2* carriers report no family history of breast or ovarian cancers. Therefore, the guidelines for family history used for Caucasians in recommending genetic testing may miss a significant percentage of African American *BRCA* carriers.
- Considering all data, fifty-nine percent of pathogenic *BRCA1* mutations (protein truncating, disease-associated missense, splicing) and 50% of *BRCA2* mutations are unique to patients of African ancestry. Only 26% of the pathogenic *BRCA1* and 17% of the pathogenic *BRCA2* mutations have been identified in more than family of African ancestry. Therefore, African Americans exhibit a unique broad spectrum of *BRCA1* and *BRCA2* mutations.
- The importance of this work is for genetic testing and genetic counseling. The numerous, distinct pathogenic mutations in *BRCA1* and *BRCA2* observed in African Americans reflects

the high level of genetic diversity in people of African ancestry. Because of this broad spectrum of distinct mutations, genetic testing for *BRCA1* and *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients. It is noteworthy that *BRCA* mutations were detected in individuals without a reported history of disease among early onset (≤40 years) breast cancer patients, male breast cancer patients, and a patient with both breast and ovarian cancers.

REFERENCES

Breast Cancer Information Core (BIC) [web site] http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

Castilla LH, Couch FJ, Erdos MR, Hoskins KF, Calzone K, Garber JE, Boyd J, et al. (1994) Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. Nat Genet 8:387-391

Easton DF (1994) The inherited component of cancer. Br Med Bull 50:527-535

Eeles RA, Stratton MR, Goldgar DE, Easton DF (1994) The genetics of familial breast cancer and their practical implications. Eur J Cancer 30A:1383-1390

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT et al. (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676-689

Frank TS, Manley SA, Olopade OI, Cummings S, Garber JE, Bernhardt B, Antman K et al. (1998) Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. J Clin Oncol 16:2417-2425

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tasvtigian S, Bennett LM, et al. (1994) BRCA1 mutations in primary breast and ovarian carcinomas. Science 266:120-122

Gao Q, Tomlinson G, Das S, Cummings S, Sveen L, Fackenthal J, Schumm P, Olopade OI (2000) Prevalence of *BRCA1* and *BRCA2* mutations among clinic-based African American families with breast cancer. Hum Genet 107:186-191

Jorde LB, Bamshad M, Rogers AR (1998) Using mitochondrial and nuclear DNA markers to reconstruct human evolution. Bioessays 20:126-136

Kanaan, Y., Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000a) BRCA2 mutations in African Americans. Poster presentation, American Society of Human Genetics, Philadelphia, October, 2000. Am. J. Hum. Genet. (supplement 2) 67:92 abs. #455

Kanaan, Y., Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., Whitfield-Broome, C. (2000b) BRCA2 mutations in African Americans. Poster presentation, Howard University, Graduate School of Arts and Sciences, April, 2000.

Kanaan, Y., Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., Whitfield-Broome, C. (2000c) BRCA2 mutations in African Americans. Oral & poster presentation, Howard University Medical Center Scientific Forum, May, 2000.

Kanaan, Y., Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000d) BRCA2 mutations in African Americans. Oral & poster presentation, American Society for Microbiology, Washington D.C. branch, student meeting, May, 2000

Kanaan, Y., Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C**. (2000e) BRCA2 mutations in African Americans. Oral & poster presentation, National Medical Association, August, 2000.

Krainer M, Silva-Aarrieta S, FitzGerald MG, Shimada A, Ishioka C, Kanamaru R, MacDonald DJ et al. (1997) Differential contributions of BRCA1 and BRCA2 to early-onset breast cancer. N Engl J Med 336:1416-1421

Mefford HC, Baumbach L, Panguluri RCK, **Whitfield-Broome C**, Szabo C, Smith S, King MC, Dunston G, Stoppa-Lyonnet D, Arena F. (1999) Evidence for a BRCA1 founder mutation in families of west African ancestry. Am J Hum Genet 65:575-578.

Miki Y, Swenson J, Shattuck-Eidens D, Futreal PA, Harshman K. Tavitigian S, Liu Q, et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71, 1994

Olopade, O., Dunston, G., Tainsky, M., Collins, F., **Whitfield-Broome. C.** (2000) Breast cancer genetics. Invited presentation, Summit meeting evaluating research on breast cancer in African American women, Washington D.C., September, 2000. Manuscript in preparation.

Panguluri RCK, Brody LC, Modali R, Utley K, Adams-Campbell L, Day AA, **Whitfield-Broome C,** & Dunston GM. (1999) BRCA1 mutations in African Americans. Human Genetics 105:28-31 and on line http://dx.doi.org/10.1007/s004399900085.

Ries LAG, Kosary CL, Hankey BF, Miller BA, Harras A, Edwards BK (1998) Surveillance, Epidemiology, and End Results (SEER) Program [web site] Bethesda MD: National Institutes of Health, http://www.seer.ims.nci.nih.gov/

Wagner TMU, Hirtenlehner K, Shen P, Moeslinger R, Muhr D, Fleischmann E et al. (1999) Global sequence diversity of *BRCA2*: analysis of 71 breast cancer families and 95 control individuals of world wide populations. Human Mol Genet 8:413-423

Whitfield-Broome C, (1999) Inheritance of Breast Cancer in African American Women: How Should We Monitor? Presentation, Howard University Women's Health Institute, Washington DC, April, 1999. The Proceedings of Health Issues and Concerns of Women of Color: A Call to Action, Health and Human Services, in press.

Whitfield-Broome, C., Dunston, G.M., Brody, L.C. (1999) American Association for Cancer Research, Philadelphia, April, 1999 BRCA2 Mutations in African Americans, Proc. Amer. Assoc. for Cancer Research, 40:269, abstract #1788

Whitfield-Broome, C., Kanaan, Y., Kpenu, E., Utley, K., Dunston, G.M., Brody, L.C. (2000) BRCA2 mutations in African Americans. Poster presentation, Era of Hope Department of Defense Breast Cancer Research Program Meeting, Atlanta, GA., June, 2000.

APPENDICES

Table 1 African American breast cancer families at high risk for breast cancer predisposing mutations

Case category for selection	Number of cases (% total cases)
Multiple-case families (≥3[a])	35 (47%)
Multiple-case families (2[a])	26 (35%)
Early Onset (≤ 40 years) breast cancer, no family history	9 (12%)
Bilateral breast cancer	1 (1.5%)
Male breast cancer	2 (3%)
Breast and ovarian cancers[b]	1 (1.5%)
Total cases	74 (100%)
Multiple-case families ≥3 with ovarian ca	5 (7%)
Multiple-case families ≥6	8 (11%)
Cases tested ≤ 40 years	30 (41%)
New samples collected, not tested	
Multiple-case families (≥3[a])	2 (33%)
Multiple-case families (2[a])	1 (17%)
Early Onset (≤ 40 years)	3 (50%)
Total cases	6 (100%)
Cases ≤ 40 years	3 (50%)

[a]Number of breast cancer and ovarian cases per family, including first-degree, second-degree, and distant relatives.

[b]Same individual with both breast and ovarian cancers.

Table 2 *BRCA1* sequence alterations in African American breast cancer patients. (*BR*, breast; number in *parentheses* indicates number of breast and ovarian cancer cases per family)

Case number	Cancer type	Age at diagnosis (years)	Exon	Nucleotide[a] /codon	Mutation	Amino acid change	Designation
Pathogenic, protein truncating mutation							
BC053	BR(4)	55	11	2495	Insertion G	Frameshift stop at codon 800[b]	2495insG
Polymorphisms (≥1% of chromosomes in this or other studies) and **Noncoding variations**							
BC048	BR(2)	40	11	1256/379	T to G	Ile to Met	I379M
BC051	BR(2)	38	11	1256/379	T to G	Ile to Met	I379M
BC052	BR(2)	33	11	1186/356	A to G	Gln to Arg	Q356R
			11	2576/819	C to A	Ser to Tyr	S819Y
			Intron 7	-	C to T	Non-coding	IVS7+553C/T
BC054	BR(6)	76	11	2576/819	C to A	Ser to Tyr	S819Y

[a]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA1* cDNA (GenBank accession no. U14680).
[b]Including newly inserted/deleted amino acids and stop codon.

Table 3 *BRCA2* pathogenic mutations and missense variations in African American breast cancer patients.

Case number	# Cancers per family[a]	Age at diagnosis (years)	Exon	Nucleotide[b] /codon	Mutation	Amino acid change[c]	Designation
Pathogenic Mutations							
BC078	6 BR 1 St	43	10	1882/552	deletion T	Frameshift, stop at codon 557	1882delT[d]
BC021 male	1 male BR, 1female	44	10	1991/588	deletion ATAA	Frameshift, stop at Codon 612	1991del4[e]
BC076	7 BR 1OV	Bilateral 37 BR, 45 BR	10	1993/589	deletion AA	Frameshift, stop at codon 595	1993delAA
BC051	2 BR	38	10	2001/591	deletion TTAT	Frameshift Stop at codon 612	2001del4[e]
BC029 male	1 male Br, 3 Pr, 1Lu	59	11	2816/863	insertion A	Frameshift, stop at Codon 880	2816insA[e]
BC061 OV	3 BR 2 OV	44	11	4075/1283	deletion GT	Frameshift stop at Codon 1284	4075delGT[d]
BC002	Early Onset	37	11	4088/1287	deletion A	Frameshift, stop at Codon 1292	4088delA[d]
BC001	Early Onset	33	19	8643/2805 8642/2805	deletion AT, 8642 T to C	Frameshift stop at codon 2810	8643delAT[d]

[a]BR breast; Lu, lung; OV ovarian; Pr, prostate; St, stomach.
[b]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA2* cDNA (GenBank accession no. U14680).
[c]Including newly inserted/deleted amino acids and stop codon.
[d]Kanaan et al., 2000a.
[e]Whitfield-Broome et al., 1999.

Table 4 *BRCA2* rare variations in African American breast cancer patients[a,b]

Case number	# Cancers per family[c]	Age at diagnosis (years)	Exon/ Intron	Nucleotide[d]/ codon	Mutation	Effect[e]	Designation
BC011	3 BR	57	ex 14	7412/2395	A to T	His to Leu	H2395L
BC049	3 BR 2 Co	67	ex 2	214	A to C	Noncoding 5' UTR	214A>C
BC038	2 BR	68	ex 3	459/77	T to G	Silent, thr	T77T
BC018	1 BR bilater-al	59	in 7	IVS7 + 611	deletion CTTAA	noncoding	IVS7 + 611 del5
BC023	3 BR	63	in 26	IVS26 + 24	A to G	noncoding	IVS26 + 24A>G
				IVS26 + 133	T to G		IVS26 + 133T>G

[a]Rare variants are those occuring with a frequency of <1 out of 100 chromosomes.
[b]Kanaan et al., 2000a.
[c]BR breast; Co, colon; Pr, prostate.
[d]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA2* cDNA (GenBank accession no. U14680).
[e]IVS, intervening sequence; +, number of nucleotides into the intron; intron 7 is 3' to exon 7.

Table 5 . *BRCA2* polymorphisms in African American breast cancer patients[a,b]

Exon Intron	Nucleotide change[c]	Effect	Variant Allelic Frequency[d]
ex 2	203 G to A	noncoding 5' untranslated region	0.15 (18/120)
ex 2	218 C to T	noncoding 5' untranslated region	0.017 (2/120)
in 12	IVS12 + 3 deletion T	noncoding (not splicing)	0.048 (6/126)
ex 14A	7470 A to G	silent, Ser 2414	0.083 (10/120)
ex 14B	7625 C to T	Ala 2466 Val	0.020 (2/102)
in 14B	IVS14 + 53 C to T	noncoding	0.029 (3/102)
in 15	IVS15 + 5 C to T	noncoding	0.018 (2/114)
in 21	IVS21 + 495 T to C	noncoding	0.448 (52/116)

[a]Polymorphisms, variants occuring with a frequency of ≥1 out of 100 chromosomes.
[b]Kanaan et al., 2000a.
[b]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA2* cDNA. IVS, intervening sequence; +, number of nucleotides into the intron; intron 12 is 3' to exon 12.
[c]The variant allelic frequency was determined by dividing the variant chromosomes by the total chromosomes (numbers in parentheses).

African American Breast Cancer Summit Position Paper ***DRAFT***

September, 2000
Olufunmilayo Olopade, Georgia Dunston, Michael Tainsky, Francis Collins, Carolyn Broome

Breast Cancer Genetics

As improved treatment of cancer has become a reality leading to increased survival of patients with diverse types of tumors, the emphasis on cancer control through prevention and early detection is growing. Unfortunately, breast cancer mortality continues to rise among young black women. A clear understanding of associated risk factors will likely lead to the development of novel interventions to prevent breast cancer and drastically reduce mortality from the disease. One of the most promising areas of research is the identification of gene-environment or gene-gene interactions that lead to increased breast cancer risk among young women. As was expected, the Human Genome Project has yielded significant new knowledge about breast cancer susceptibility genes such as *BRCA1* and *BRCA2* genes. Genetic testing for *BRCA1* and *BRCA2* genes has become a reality, moving from the research setting into clinical practice. To ensure equity and justice in the clinical use of genetic testing, an understanding of the unique needs and concerns of special populations is necessary. It is imperative that breast cancer genetic studies include individuals of all ethnic backgrounds so that the benefits of this new technology can be enjoyed by all! This paper reviews the state of the technology for African American women and identifies opportunities for research into the genetic bases of breast cancer among African American women.

Comment on the parallels between BRCA-related cancers and breast tumors among African Americans (younger age distribution, higher rates of ER negativity, more anaplastic lesions, more medullary lesions)

Several epidemiological and histopathological observations reveal striking similarities between BRCA related breast cancers and breast cancers that occur in African Americans. BRCA-associated breast cancers occur at an earlier average age than sporadic breast cancers. Similarly, it has been observed that African-American patients have a greater breast cancer incidence between 30-49 years than Whites (Ries et al., 1999). Additionally, BRCA-associated breast and ovarian cancers are characterized by higher than expected frequencies of medullary or atypical medullary carcinoma, high tumor grade, hormone receptor negativity, and p53 mutation (Phillips et al., 1999). Tumor cells from women with a high likelihood of carrying a BRCA mutation have a higher than expected frequency of aneuploidy, S-phase fraction as well as hormone receptor negativity (Marcus et al., 1996; Watson et al., 1998). Tubular and lobular cancers are not common among *BRCA1* mutation carriers but are more common among *BRCA2* mutation carriers (Marcus et al., 1996). Likewise, tumors in young African-American women are more likely to be poorly differentiated, and estrogen receptor negative, and to exhibit high nuclear atypia, and higher S-phase (Ijaduola and Smith, 1998; Weiss et al., 1995). The greater percentage of Black women than White women diagnosed with breast cancer under age 50 and the histopathological similarities between BRCA-associated cancers and cancer occurrence in African Americans suggests a genetic contribution to breast cancer in African-American women. However, very few data are available from this population to evaluate this possibility.

What is the incidence of currently-known deleterious BRCA mutations among African Americans?

***BRCA1* Mutations**

Although a few African American families were included in the earliest studies of *BRCA1* mutations, most studies have focused on Caucasian women with a strong family history of breast cancer. Pathogenic *BRCA1* amino acid substitutions (missense mutations M1775R, C64G), which segregate with the disease in large African American families and occur in functionally important regions of the protein were first described (Miki et al., 1994; Futreal et al., 1994; Castilla et al., 1994, Monteiro et al., 1996; Table 1). *BRCA1* protein truncating, frameshift mutations were initially reported by Arena et al. (1996) Gao et al. (1997) and Stoppa-Lyonnet et al. (1997) (Table 1). Three frameshift mutations were identified by Arena et al. (1996) in Florida African Americans with a strong family history of the disease. Stoppa-Lyonnet et al. (1997) and Arena et al. (1996) detected the same 10 base pair duplication (943ins10) in families from the Ivory Coast and Florida. Five protein truncating *BRCA1* mutations were identified in nine high-risk families (56%) by Gao et al., 1997. These *BRCA1* mutations were found in early-onset breast and/or ovarian cancer families with average ages at diagnosis below 40 years. Four of the five *BRCA1* mutations were also identified in families with three or more cases of breast and/or ovarian cancer. In a study of 85 African American breast cancer patients, one additional *BRCA1* frameshift mutation (1625del5) was discovered by Gao et al., 2000b.

Ganguly et al. (1998) reported two deleterious *BRCA1* mutations among 10 African American patients with a family history of breast cancer. Panguluri et al. (1999) identified 2 deleterious *BRCA1* mutations, 943ins10 and 3450del4, among 45 high-risk African American families, selected for family history, early age of onset, breast and ovarian cancer, bilateral breast cancer, and male breast cancer. Shen et al. (2000) examined exons 2, 5, 11, 16, and 20 of the *BRCA1* gene in 54 African American breast cancer patients, not selected for family history or age, and found one novel frameshift mutation. Numerous mutations in *BRCA1* have been reported to the Breast Cancer Information Core (Bic) by Myriad and other investigators (Table 1).

In a population based case-control study in North Carolina, white women exhibited a higher frequency of *BRCA1* mutations than black women (Newman et al. 1998). No disease-related *BRCA1* mutations were identified in 88 black women with breast cancer; three pathogenic *BRCA1* mutations were identified in 120 white women with breast cancer. Family history and age of onset were not delineated by race for the cases. A polymorphism in the 3' untranslated region of *BRCA1,* which is in linkage disequilibrium with a polymorphism in intron 22, was detected at a significantly higher frequency in African American cases than in black controls.Of the 26 distinct *BRCA1* pathogenic mutations (protein truncating, disease associated missense, splicing) detected in African Americans or Africans, 58% (15) are unique to this group (Table 1). Twenty-three percent (6/26) of the pathogenic mutations have been detected in more than one family of African ancestry. Only two (8%) deleterious mutations, M1775R and 943ins10, are carried by more than two families. Therefore, African Americans exhibit a unique broad spectrum of *BRCA1* mutations and variations (Ganguly et al., 1998; Panguluri et al., 1999; Gao et al., 2000b).

***BRCA2* Mutations**

The *BRCA2* gene, which is twice as large as *BRCA1*, has been studied in African Americans less than *BRCA1*. A recurrent *BRCA2* frameshift mutation, 2816insA, has been identified in African American women with breast cancer (Gao et al., 1998, 2000). The same mutation has been detected in a male breast cancer patient by Whitfield-Broome et al., 1999. Ganguly et al. (1998) reported three disease-related *BRCA2* mutations among 10 African American patients with a family history of breast cancer (Table 2).

To examine the contributions of *BRCA1* and *BRCA2* mutations in African American women with breast cancer, Olopade and her colleagues have screened 85 African American breast cancer patients; 20 of whom were selected because of a family history of breast and/or ovarian cancer among first degree relatives (Gao et al. 2000b). The cases were ascertained in oncology clinics from three different geographic locations in the U.S and represent the largest study to date, of clinic-based population of African American women undergoing *BRCA1* and *BRCA2* mutation screening. Deleterious *BRCA1* and *BRCA2* mutations were identified in 5 patients or 6% of the entire cohort. Only 8% (1/13) of women with a family history of breast cancer, but not ovarian cancer, had mutations. However, the mutation rates were higher for women from families with a history of breast cancer and at least one ovarian cancer (3/6, 50%) or a history of breast cancer and other types of cancers (5/12, 42%). The spectrum of mutations was unique in that one novel *BRCA1* mutation (1625del5) and three novel *BRCA2* mutations (1536del4, 6696delTC and 7795delCT) were identified. Subsequently, the 7795delCT mutation was reported in another African American family and the 6696delTC mutation in 2 families of unknown ethnicity (Bic). No recurrent mutations were identified in this cohort but one *BRCA2* (2816insA) mutation had been previously reported (Gao et al., 1998). In addition, six missense mutations of unknown significance were identified; one of which was novel (Table 2). Of note, all five deleterious mutations were identified in women with significant family histories of breast and ovarian cancers, or other cancers such as pancreatic, lung and prostate cancers suggesting that these BRCA mutations are highly penetrant in African American families. In addition, one deleterious *BRCA2* mutation was identified in the 65 women (1.5%) who were unselected for age or family history; this individual reported a family history of unconfirmed cancers. Thus, among this relatively small cohort of African American women with breast cancer and a family history of the disease, the proportion with BRCA mutations is quite high at 42%.

In a study of 75 high-risk African American breast cancer patients, the Howard University/NHGRI group identified 8 pathogenic, *BRCA2* frameshift mutations after examination of the entire coding and flanking sequences (Table 2; Whitfield-Broome et al., 1999; Kanaan et al., 2000). A *BRCA2* missense variant of unknown functional significance, numerous polymorphisms, and noncoding variants were also observed in the *BRCA2* gene. One-half of the pathogenic mutations were unique to African Americans and one-half were observed in women below the age of 40, with or without a family history of disease. Two pathogenic mutation carriers were males. Four distinct disease-related *BRCA2* mutations in African Americans have been reported by Myriad (Bic).

In a population based case-control study in North Carolina, white women exhibited a 3 times higher frequency of *BRCA2* mutations than black women (Mu et al., 1999). One disease-related *BRCA2* mutation was identified in 88 black women with breast cancer. In a world-wide study of 71 breast cancer families and 95 controls, Wagner et al. (1999) did not identify any deleterious *BRCA2* mutations as occurring in African American or African families. Consistent

with the greater genetic diversity observed in people of African ancestry, a higher frequency of sequence variations were found in Africans than in other world-wide populations.

Of the 18 distinct pathogenic *BRCA2* mutations detected in families of African ancestry, 56% (10/18) are unique to this group (Table 2). Seventeen percent (3/18) of the pathogenic mutations have been detected in more than one African American or African family. Only one (6%) deleterious mutation, 2816insA, is carried by more than two families. This distribution of *BRCA2* mutations is very similar to that for *BRCA1*. Thus, African Americans have a unique mutation spectrum in the *BRCA1* and *BRCA2* genes but recurrent mutations are likely to be more widely dispersed in the African Diaspora and therefore not readily identifiable in this population (Ganguly et al., 1998; Panguluri et al., 1999; Gao et al., 2000b; Kanaan et al., 2000). For these reasons, more African American families need to be investigated for mutations and genetic testing should involve the entire coding and flanking sequences.

What founder mutations have been identified among African Americans, with a comment on the parallels between the impact of breast cancer on African Americans and native Africans (lower overall incidence, younger age distribution)

When the same mutation is found in multiple unrelated families, this may be due to ancestry from a small isolated group of founders or to independent mutational events. A common haplotype among unrelated families around the gene of interest is evidence for a founder effect. The length of the common haplotype is inversely related to the age of the mutation. The *BRCA1* 943ins10 mutation was associated with a single haplotype in 5 families from Ivory Coast, Washington DC, Florida, South Carolina, and the Bahamas (Mefford et al., 1999). The length of the common haplotype is about the same as the Ashkenazi Jewish founder mutation, 185delAG, which has been estimated to be 760 years old (Neuhausen et al., 1996). Therefore, the *BRCA1* 943ins10 mutation appears to be an ancient founder mutation of West African origin. A common haplotype was reported for two African American families with the *BRCA1* 5296del4 mutation and for two African American families with the *BRCA1* 1832del5 (Gao et al., 1997). As shown in Tables 1 and 2, *BRCA* mutations detected in African Americans or Africans have also been reported multiple times in families with and without African ancestry, but haplotyping is needed to determine if they represent founder mutations unique to African Americans.

In populations indigenous to the African tropics, breast cancer has been considered to be a rare disease, predominantly afflicting young women. However, the International Agency of Research on Cancer Bulletins and surveys in seven African countries has shown that breast cancer incidence increased from 15.3 per 100, 000 in 1976 to 33.6 per 100, 000 in 1998. Incident rates for Whites and Blacks in the United States are 118 and 103 per 100,000 respectively. The rising incidence in Africa has been attributed to increased reporting and the adoption of a western lifestyle in urban cities. In a recent review of breast cancer cases from the University of Ibadan, Nigeria, the average age at diagnosis was 42.6 years, 10-15 years younger than in Whites (Adebamowo et al.1999). The young average age at diagnosis could be partly explained by the low mean age of the general African population. However, multiple studies in the United States (U.S) have also documented a higher breast cancer incidence and death rate in pre-menopausal Black women compared to non-Hispanic Whites (http://www-seer.ims.nci.nih.gov). Thus, it is likely that the shared genetic background of Africans and U.S Blacks contributes to the greater susceptibility to early onset breast cancer in both groups but this has not been carefully examined. In the first study of it's kind, the entire coding regions as well as the intron/exon

boundaries of *BRCA1* and *BRCA2* have been examined in 70 African breast cancer patients under 40 years of age (Gao et al, 2000a). These patients were ascertained at the University of Ibadan College of Medicine, Nigeria and were not selected for a family history of breast cancer. In fact, the majority of patients in the cohort reported no family history of breast cancer, indicating that the results presented below would have been missed had the subjects been selected for family history. In this cohort, two *BRCA1* truncating mutations, four *BRCA1* missense mutations, one *BRCA2* truncating mutation and five *BRCA2* missense mutations were identified (Tables 1, 2). The truncating *BRCA1* mutation Q1090X has never been described previously and was not seen outside of one family identified in this cohort. The 1742insG mutation is also unique to this cohort. The *BRCA1* missense alleles, however, have all been described in other populations: alleles E1038G and K1183R have both been described as benign polymorphisms, and I379M and K820E have both been described as unclassified variants (Bic). The *BRCA2* truncating mutation 3034del4 has previously been described as a mutational hotspot (Neuhausen et al., 1998). The *BRCA2* missense mutations G3212R and N1880R have never been reported previously. The alleles A248T, N987I, and L929S have all been reported previously and are listed as unclassified variants.

What do we know about familial cancer syndromes that are prevalent among African Americans?

Little information exists about other familial cancer syndromes unique to African-Americans but two African-American families with Cowden's syndrome have been reported (Fackenthal et al, 2000). The same germline p53 coding mutation and haplotype were detected in two Li-Fraumeni African American families, one of which exhibited primarily breast and ovarian cancer (Hung et al., 1999).

How can we best identify African American families that may benefit from genetic testing?

Genetic counseling translates basic scientific advances into a practical and understandable form of information for the patient. It involves the collection of medical and family information, recognition of familial syndromes based on pedigree analysis, calculation of risk estimates, and effective communication of risk status at a level that the patient can understand. The National Society of Genetic Counselors has developed guidelines that counselors should follow (Kessler 1979; NSGC 1983). These include respect for autonomy and privacy of the individual, the need for confidentiality and informed consent, and the provision of information to the patient in a nondirective manner (Bennett et al., 1995, Fine et al., 1996). Most importantly, the patient should be educated about cancer prevention practices. As cancer risk assessment moves from the research setting into clinical practice, genetic counseling and patient education must be an integral part of such programs. To ensure equity and justice in the clinical use of genetic testing, an understanding of the unique needs and concerns of special populations is necessary.

It well known that the majority of clients who use genetic counseling and testing options even when covered by third party payers, are white women from socioeconomically advantaged circumstances (Lippman, 1991; March of Dimes, 1990). It has long been recognized that minority and economically disadvantaged patients do not participate in clinical trials (McCabe et al., 1994). This situation has been attributed to a number of reasons including: ignorance of the availability, fear and distrust, perception of cost, access and transportation, manner of information presentation, lack of valid, culturally sensitive questionnaires and language of

consent forms (Fleetwood, 1993; Palos, 1994; Back and Ades, 1994). Although data are limited, anecdotal reports suggest that minorities are also less likely to use cancer genetics services unless a major outreach effort is directed towards their inclusion.

The University of Chicago Cancer Risk Clinic (CRC) has made a major commitment to provide avenues for women of all socioeconomic background to take advantage of recent advances in cancer genetics. Through the CRC, we have identified more than 700 breast cancer high risk families. In 1996, the group initiated studies to determine the incidence of *BRCA1* and/or *BRCA2* mutations among individuals from ethnically diverse high risk families in collaboration with MYRIAD Genetic Laboratories (Skolnick et al., 1997). We provided pre and post-test counseling. Complete data are available on 81 individuals (representing 81 unrelated families) including 55 Caucasians of Northern European descent, 9 African Americans, 8 Ashkenazi Jewish, 5 Hispanics, and 4 Asian-Pacific. Thirty six percent (29/81) of those tested were found to have a deleterious mutation in *BRCA1*; fourteen percent (10/69) were found to have a deleterious mutation in *BRCA2*. Overall, in this ethnically diverse cohort, genetic susceptibility to breast cancer could be explained by *BRCA1* or *BRCA2* mutations in 48% (39/81) of families (Olopade et al., 1997). Mutations in the non-coding region or in other as yet unidentified genes may account for the remainder. We identified mutations in six of the nine (67%) African American families tested including two novel recurrent mutations previously unreported in Caucasians (Gao et al., 1997). These data suggest that African American families with a high incidence of breast and ovarian cancer are just as likely as Caucasian families to harbor deleterious BRCA mutations. In this study, women who developed breast cancer under the age of 50 years and have any relatives with breast cancer or ovarian cancer have at least a 1 in 5 chance of carrying an altered *BRCA1* or *BRCA2* gene. Many of these women, having survived breast cancer are at increased risk of subsequent ovarian cancer or a second primary breast cancer. Thus, a substantial percentage of young African American breast cancer survivors in the United States could be at risk for second cancers because of genetic predisposition. We should intensify our efforts to integrate genetic counseling and testing into the clinical care of young African American women already affected by cancer. Index breast cancer cases could provide the link to other at risk family members. Access to genetic testing may help minority women from such high risk families develop better strategies to reduce their risk of dying from breast or ovarian cancer.

In a companion study, we conducted 3 focus group sessions to evaluate the informational needs of African Americans participating in genetic testing trials. Most participants felt African American culture speaks to the situation of genetic testing with a unique voice -- that African Americans' collective history brings up a set of issues related to cancer prevention, treatment and medical research in general, that is distinct from Euro-American issues. At the same time, participants acknowledged the universality of the human experience with cancer, and the desire to be treated like everyone else. Conclusions and Recommendations from the focus group studies include:

- Provide a forum where African Americans with family histories of cancer can discuss issues related to cancer.
- Use a local spokesperson or community leader who is African American, a cancer survivor, or has participated in genetic testing to speak at community centers.
- Develop a media campaign using a celebrity spokesperson who is African American, has had cancer or has a family history of cancer.

- Provide a toll-free or local phone number for people to call with any questions about genetic testing.
- Use community centers and high traffic areas such as churches, public transportation, schools, park district centers, and libraries to distribute written information and post the toll-free or local phone number.

An intensive outreach program focused on cancer control through genetics could improve awareness of genetics in the African American community.

Are genetic counselors likely to be sensitive to cultural/ethnic issues?

Compared to Caucasian women, African American women had lower levels of knowledge and had more positive attitudes about the benefits of genetic testing (Hughes et al. 1999). There were no significant ethnic differences in attitudes about the limitations and risks of testing, however, income was negatively associated with this outcome. Ethnic differences in knowledge and attitudes about genetic testing for breast-ovarian cancer risk may be attributable to differences in exposure to genetic information and referral by health care providers.

Little information exists about rates or predictors of test use among African Americans. In a few studies that have examined rates of test use among individuals from high-risk families who have self-referred for genetic counseling/testing, it appears that spiritual faith and psychological factors influenced testing decisions. Lerman and her colleagues have recently published their findings in 290 (including African American) women with familial breast cancer who were offered genetic counseling and testing for alterations in the *BRCA1* and *BRCA2* genes (Schwartz et al. 2000). Baseline levels of spiritual faith, cancer-specific distress, perceived risk, and demographic factors were examined to identify independent predictors of whether participants received versus decline testing. Among women who perceived themselves to be at low risk of developing breast cancer again, those with higher levels of spiritual faith were significantly less likely to be tested, compared with those with lower levels of faith (OR, 0.2; 95% CIs, 0.1 and 0.5). However, among women with high levels of perceived risk, rates of test use were high, regardless of levels of spiritual faith (OR, 1.2; 95% CIs, 0.4 and 3.0). These results highlight the role that spirituality may play in the decision-making process about genetic testing. Unfortunately, in our experience, we have found that African American women generally underestimate their risk and this correlated with higher levels of spiritual faith. In fact, despite extensive genetic counseling, cancer risk perception among African-American women remains closely associated with personal experiences (ASCO abstract—Cummings). Consideration of these factors may be important in effectively design risk assessment and education programs for minority women.

Conclusions

It is important to study more African American and African families with *BRCA1* and *BRCA2* mutations in order to (1) assess prognosis and treatment in carriers, (2) investigate tumor pathology, estrogen status, p53 levels, HER2/Neu levels in carriers, (3) study other breast cancer predisposing genes or polymorphisms in *BRCA* carriers without disease, (4) determine who will most benefit from genetic testing, and (5) measure penetrance. Since gene-gene interactions are likely to be significant in both sporadic and inherited cancer, germline polymorphisms and other genetic variations need to be studied. Investigation of a much larger number of *BRCA* mutations in African Americans and Africans will promote observations and conclusions that are significant.

References

Adebamowo, C.A., and O.O. Adekunle. 1999. Case-controlled study of the epidemiological risk factors for breast cancer in Nigeria. *British Journal of Surgery*. 86:665-668.

Arena, J., L. Baumbach, S. Smith, L. Gayol, E. Perera, and H. Lubs. 1998. *BRCA1* mutation analysis in 20 at-risk African-American families supports a low frequency of germ-line mutation. *The American Journal of Human Genetics*. 63 (Supplement):A62.

Arena, J.F., S. Smith, M. Plewinska, L. Gayol, F. Villegas, E. Perera, P. Murphy, and H. Lubs. 1996. *BRCA1* mutations in African American women. *The American Journal oh Human Genetics (Supplement)*. 59:A34.

Arena, J.F., S. Smith, V. Vincek, L. Gayol, F. Villegas, E. Perera, M. King, C. Szabo, A. Restrepo, and H. Lubs. 1997. A *BRCA1* founder mutation in African Americans. *The American Journal of Human Genetics (Supplement)*. 61:A14.

Back, B., and T. Ades. 1994. American Cancer Society Urban Demonstration Projects: Models for Successful Intervention. *Semin Oncol Nurs*. 10:96-103.

Bennett, R.L., K.A. Steinhaus, S.B. Uhrich, C.K. O'Sullivan, R.G. Resta, D. Lochner-Doyle, D.S. Markel, V. Vincent, and J. Hamanishi. 1995. Recommendations for standardized human pedigree nomenclature. *J Genet Counsel*. 4:267-280.

Breast Cancer Information Core (BIC) (1998) [web site] http://www.nhgri..nih.gov/Intramural_research/Lab_transfer/Bic/

Castilla LH, Couch FJ, Erdos MR, Hoskins KF, Calzone K, Garber JE, Boyd J, et al. 1994. Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. *Nature Genetics* 8:387-391

Dangel, J., J. Wagner-Costalas, B. Bove, L. Vanderveer, M. Itzen, M. Daly, and A.K. Godwin. 1999. Novel germline *BRCA1* mutation (155del4) in an African American with early-onset breast cancer. *Human Mutation*. 14:545.

Gayol, L., T. Schol, H. Basterrechea, I. Pfeifer, J. Davies, E. Perera, S. Smith, J.F. Arena, L. Baumbach. 1999. BRCA1 mutation analysis in at-risk African-American families: results and implications. *The American Journal of Human Genetics (Supplement)*. 65:A127.

Fackenthal, J., D. Marsh, A. Richardson, C. Eng, S. Cummings, B. Robinson, and O. Olopade. in preparation. Male breast cancer is associated with Cowden Syndrome patients with germline PTEN mutations.

Feigelson H.S., A.T. Diep, C.L. Pearce, K.R. Monroe, N. Zhou, R.W. Haile, and B.E. Henderson (1998) BRCA1 missense changes among African-American and Latina women. *Proceedings of the American Association for Cancer Research*, 39:475, abstract #3229

Fine, B., D. Baker, M. Fiddler, and e. al. 1996. Practice-based Competencies for Accreditation of Training in Graduate Programs in Genetic Counseling. *J Genet Counsel*. 5:113-122.

Fleetwood, M. 1993. Clinical Trials and Cultural Diversity. *Oncology Issues*. (Summer):19-22.

Futreal, P.A., Q. Liu, E.D. Shattuck, C. Cochran, K. Harshman, S. Tavtigian, L.M. Bennett, S.A. Haugen, J. Swensen, Y. Miki, and et al. 1994. *BRCA1* mutations in primary breast and ovarian carcinomas. *Science*. 266:120-122.

Ganguly, T., M. Citron, J. Stott, C. Isaacs, B. Peshkin, L. Godmilow, B. Weber, and A. Ganguly. 1998. Novel BRCA mutations in African American individuals with breast and ovarian cancer. *The American Journal of Human Genetics (Supplement)*. 63:A69.

Gao, Q., C. Adebamowo, J. Fackenthal, S. Das, L. Sveen, A. Falusi, and O. Olopade. 2000a. Protein truncating *BRCA1* and *BRCA2* mutations in African women with pre-menopausal breast cancer. *Human Genetics*. DOI 10.1007/s004390000342.

Gao, Q., S. Neuhausen, S. Cummings, M. Luce, and O.I. Olopade. 1997. Recurrent germ-line *BRCA1* mutations in extended African American families with early-onset breast cancer. *American Journal of Human Genetics*. 60:1233-1236.

Gao, Q., L. Sveen, S. Cummings, S. Turner-Thompson, G. Tomlinson, M. Dean, C. Issacs, and O. I.Olopade. 1998. Contribution of recurrent BRCA1 (B1) AND brca2 (B2) mutations to breast cancer in African American (AA) women., *Proceedings of the American Association for Cancer Research*, 39:475, abstract #3229

Gao, Q., G. Tomlinson, S. Das, S. Cummings, L. Sveen, J. Fackenthal, P. Schumm, and O. Olopade. 2000b. Prevalence of *BRCA1* and *BRCA2* mutations among clinic-based African American families with breast cancer. *Human Genetics*. DOI 10.1007/s004390000290.

Hughes C, Gomez-Caminero A, Benkendorf J, Kerner J, Isaacs C, Barter J, Lerman C. 1997 Ethnic differences in knowledge and attitudes about *BRCA1* testing in women at increased risk. Patient Educ Couns 32:51-62

Hung J., Mims B., Lozano G. et al.1999. TP53 mutation and haplotype analysis of two large African American families. *Human Mutation* 14:216-221

Ijaduola, T.G., and E.B. Smith. 1998. Pattern of breast cancer among white-American, African-American, and nonimmigrant west-African women. *Journal of the National Medical Association*. 90:547-551.

Kanaan Y.M., Kpenu E., Utley K., Brody L.C., Dunston G. M., Whitfield-Broome C. 2000. BRCA2 mutations in African Americans. *The American Journal of Human Genetics (Supplement)* in press.

Kessler, S. 1979. The Psychological Foundations of Genetic Counseling. *In* Genetic Counseling: Psychological Dimensions. S. Kessler, editor. Academic Press, Baltimore. 17-33.

Lippman, A. 1991. Prenatal Genetic Testing and Screening: Constructing Needs and are Reinforcing Inequities. *Am J Law Med*. 105:11-123.

March_of_Dimes. 1990. Symposium on Genetic Services of Underserved Populations (May 1988). *Birth Defects: Original Article Series*.

Marcus, J.N., P. Watson, D.L. Page, S.A. Narod, G.M. Lenoir, P. Tonin, S.L. Linder, G. Salerno, T.A. Conway, and H.T. Lynch. 1996. Hereditary breast cancer: pathobiology, prognosis, and *BRCA1* and *BRCA2* gene linkage. *Cancer*. 77:697-709.

McCabe, M., C. Varricchio, and R. Padberg. 1994. Efforts to Recruit the Economically Disadvantaged to National Clinical Trials. *Semin Oncol Nurs*. 10:123-129.

Mefford, H.C., L. Baumbach, R.C. Panguluri, C. Whitfield-Broome, C. Szabo, S. Smith, M.C. King, G. Dunston, D. Stoppa-Lyonnet, and F. Arena. 1999. Evidence for a *BRCA1* founder mutation in families of West African ancestry [letter]. *American Journal of Human Genetics*. 65:575-578.

Miki, Y., J. Swensen, E.D. Shattuck, P.A. Futreal, K. Harshman, S. Tavtigian, Q. Liu, C. Cochran, L.M. Bennett, W. Ding, and a.l. et. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science*. 266:66-71.

Monteiro A.N.A., August A., Hanafusa H., 1996. Evidence for a transcriptional activation function of *BRCA1* C-terminal region. *Proc. Natl. Acad. Sci. USA* 93:13595-13599

Mu, H., Newman, B., Rousseau, C., Payne, S., Gordon, K., Gold, D., Tse, C-K.J., Millikan, R.C., King, M.-C. 1999. Frequency of breast cancer attriabutable to BRCA1 and BRCA2 in a population-based series of Caucasian and African-American women. *The American Journal of Human Genetics (Supplement)* 65:A21

Neuhausen, S.L., A.K. Godwin, B.R. Gershoni, E. Schubert, J. Garber, L.D. Stoppa, E. Olah, B. Csokay, O. Serova, F. Lalloo, A. Osorio, M. Stratton, K. Offit, J. Boyd, M.A. Caligo, R.J. Scott, A. Schofield, E. Teugels, M. Schwab, A.L. Cannon, T. Bishop, D. Easton, J. Benitez, M.C. King, D. Goldgar, and a.l. et. 1998. Haplotype and phenotype analysis of nine recurrent *BRCA2* mutations in 111 families: results of an international study. *American Journal of Human Genetics*. 62:1381-1388.

Neuhausen, S.L., S. Mazoyer, L. Friedman, M. Stratton, K. Offit., A. Caligo, G. Tomlinson, L. Cannon-Albright, T. Bishop, D. Kelsell and et al. 1996. Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. *American Journal of Human Genetics*. 58:271-280.

Newman, B., H. Mu, L.M. Butler, R.C. Millikan, P.G. Moorman, and M.C. King. 1998. Frequency of breast cancer attributable to *BRCA1* in a population-based series of American women. *Jama*. 279:915-921.

Olopade, O., S. Cummings, D. Malone, P. Baker, P. Schumm, P. Mumby, D. Chapman, M. Cobleigh, B. Fine, L. Esserman, D. Shattuck-Eidens, A. Oliphant, B. Ward, and M. Luce. 1997. Acceptance of *BRCA1/BRCA2* Testing is High Among Cancer Patients from High Risk Families. *In* poster presented at the ASCO meeting.

Palos, G. 1994. Cultural heritage: Cancer Screening and Early Detection. *Semin Oncol Nurs*. 10:104-113.

Panguluri, R.C., L.C. Brody, R. Modali, K. Utley, L. Adams-Campbell, A.A. Day, C. Whitfield-Broome, and G.M. Dunston. 1999. *BRCA1* mutations in African Americans. *Human Genetics*. 105:28-31.

Phillips, K.A., K. Nichol, H. Ozcelik, J. Knight, S.J. Done, P.J. Goodwin, and I.L. Andrulis. 1999. Frequency of p53 mutations in breast carcinomas from Ashkenazi Jewish carriers of *BRCA1* mutations. *Journal of the National Cancer Institute*. 91:469-473.

Ries L.A.G., Kosary C.L., Hankey B.F., Miller B.A., Clegg L., Edwards B.K. (eds) 1999. Surveillance, Epidemiology, and End Results (SEER) cancer statistics review, 1973-1997, National Cancer Institute, Bethesda, MD, http://www.seer.ims.nci.nih.gov

Shen, D., Y. Wu, M. Subbarao, H. Bhat, R. Chillar, and J.V. Vadgama. 2000. Mutation analysis of *BRCA1* gene in African-American patients with breast cancer. *Journal of the National Medical Association*. 92:29-35.

Skolnick, M., A. Oliphant, A. Shattuck-Eiden, D. Thomas, F. Teny, M. Fujimura, M. McClure, C. McBride, J. Gupta, R. Rubano, M. Staebell, K. Gumpper, S. Manley, M. Luce, B. Ward, T. Frank, L. Albright, K. Antman, R. Burgemeister, B. Bernhart, S. Buys, M. Caleffi, M. Caligo, M. Daly, M. Dosik, S. Edge, J. Garber, R. Greenstein, E. Guilotto, C. Issacs, M. Lippman, R. Michaelson, H. Navanlinna, K. Offit, T. Gilewski, L. Norton, M. Osborne, F. Strang, O. Olopade, P. Radice, M. Pierotti, A. Ravailoi, P. Rowley, R. Scott, I. Teplar, G. Tomlinson, B. Weber, E. Winer, and M. Wood. 1996. Complete DNA Sequence Analysis of *BRCA1* in 708 Women at High Risk for Susceptibility Mutations. *American Society of Human Genetics*. (Submitted).

Schwartz MD, Hughes C, Roth J, Main D, Peshkin BN, Isaacs C, Kavanagh C, Lerman C. 2000 Spiritual faith and genetic testing decisions among high-risk breast cancer probands. Cancer Epidemiol Biomarkers Prev. 4:381-5.

Stoppa-Lyonnet, D., P. Laurent-Puig, L. Essioux, S. Pages, G. Ithier, L. Ligot, A. Fourquet, R.J. Salmon, K.B. Clough, P. Pouillart, C. Bonaiti-Pellie, and G. Thomas. 1997. *BRCA1* sequence

variations in 160 individuals referred to a breast/ovarian family cancer clinic. Institut Curie Breast Cancer Group [see comments]. *American Journal of Human Genetics*. 60:1021-1030.
Sutphen, R. and T.M. Ferlita. 1999. Inherited breast/ovarian cancer in African-American. . *The American Journal of Human Genetics (Supplement)*. 65:A325.
Wagner, T.M., K. Hirtenlehner, P. Shen, R. Moeslinger, D. Muhr, E. Fleischmann, H. Concin, W. Doeller, A. Haid, A.H. Lang, P. Mayer, E. Petru, E. Ropp, G. Langbauer, E. Kubista, O. Scheiner, P. Underhill, J. Mountain, M. Stierer, C. Zielinski, and P. Oefner. 1999. Global sequence diversity of *BRCA2*: analysis of 71 breast cancer families and 95 control individuals of worldwide populations [published erratum appears in Hum Mol Genet 1999 Apr;8(4):717-9]. *Human Molecular Genetics*. 8:413-423.
Watson, P., J.N. Marcus, and H.T. Lynch. 1998. Prognosis of *BRCA1* hereditary breast cancer. *Lancet*. 351:304-305.
Weiss, S.E., P.I. Tartter, S. Ahmed, S.T. Brower, C. Brusco, K. Bossolt, J.B. Amberson, and J. Bratton. 1995. Ethnic differences in risk and prognostic factors for breast cancer. *Cancer*. 76:268-274.
Whitfield-Broome, C., Dunston, G.M., Brody, L.C. 1999. BRCA2 Mutations in African Americans, *Proceedings of the American Association for Cancer Research*, 40: 269, abstract #1788

Table 1. *BRCA1* mutations in African American and African breast cancer patients. Af Am, African American; Af, African; Af Ances, African ancestry; non-Af Ances, not of African Ancestry; FS, frameshift (identified by nucleotide, nt); N, nonsense (codon); MS, missense (codon); IVS, intervening sequence (intron #, nt) S, splicing (nt); P, polymorphism [≥1out of 100 chromosomes in breast cancer cases or controls with African ancestry or non-African ancestry]; UV, unclassified variant (amino acid substitutions are identified by codon first and by nt in parentheses; IFD, in-frame deletion (codon); UTR, untranslated region. References: 1, (Gao et al., 2000a). 2, (Gao et al., 2000b). 3, (Gao et al., 1997). 4, (Shen et al., 2000). 5, (Dangel et al., 1999). 6, (Mefford et al., 1999). 7, (Panguluri et al., 1999). 8, (Newman et al., 1998). 9, (Arena et al., 1996). 10, (Arena et al., 1997). 11, (Arena et al., 1998). 12, (Stoppa-Lyonnet et al., 1997). 13, (Stuphen et al., 1999). 14, (Bic). 15, (Ganguly et al., 1998). 16, (Gao et al., 1999). 17, (Castilla et al., 1994). 18, (Miki et al., 1994). 19, (Futreal, et al., 1994). 20, (Feigelson, et al., 1998). 21, (Gayol et al., 1999).

Table 2. *BRCA2* mutations in African American and African breast cancer patients. Abrreviations: Table 1; P*, polymorphism in 21 African controls (Wagner et al., 1999; and Bic). References as in Table 1: 22, (Whitfield-Broome et al., 1999). 23, (Kanaan et al., 2000).

Acknowledgements
Carolyn Whitfield Broome is supported by U.S. Army Medical Research and Materiel Command under DAMD17-98-8106

TABLE 1. *BRCA1* Mutations in African American and African Breast Cancer Patients

BRCA1 mutations	Effect	#Af Ances Families	¥non-Af Ances Families	Reference
Protein Truncating				
155del4	FS	1 Af Am	0	5
IVS9+GG (802insGG)	FS	1 Af Am	0	3
943ins10	FS	7 Af Am, 1 Af	2	6, 7, 9, 11-14
1625del5	FS	1 Af Am	0	2
1742insG	FS	1 Af	0	1
1832del5	FS	2 Af Am	0	3
E673X	N	1 Af Am	0	14
2418delA	FS	2 Af Am	0	14
3331insG	FS	1 Af Am	0	4
3450del4	FS	1 Af Am	9	7, 14
Q1090X	N	1 Af	0	1
3875delGTCT	FS	1 Af Am	35	11, 14
3883insA	FS	1 Af Am	0	3
3888delGA	FS	1 Af Am	0	9
K1290X	N	1 Af Am	1	14
4160delAG	FS	1 Af Am	0	9
Y1463X	N	1 Af Am	2	14
4730insG	FS	1 Af Am	0	14
4794insA	FS	1 Af Am	0	15
5296del4	FS	2 Af Am	9	3, 14
Disease Associated Missense Mutations				
C61G	MS	2 Af Am	65	16
C64G	MS	1 Af Am	2	17, 14
W1718C	MS	1 Af Am	1	14, 21
M1775R	MS	5 Af Am	7	14, 16, 18, 19
Splicing Mutations				
IVS4-1G/T	S	1 Af Am	9	14, 15
IVS13+1G/A	S	1 Af Am	0	14
Unclassified Variants and Polymorphisms				
K38K (G233A)	P	2 Af Am	2	8, 14
S186Y	UV	1 Af Am	2	2, 14
M297I	UV	1 Af Am	0	14
R315G	UV	1 Af Am	0	14
K355R	UV	1Af Am	0	14, 20
Q356R (A1186G)	P	4 Af Am	90	14, 20
I379M	UV	3 Af Am, 1Af	4	1, 8, 14
A521T	UV	1Af Am	0	14
S616delS	IFD	1 Af Am	1	14
N723D	UV	1 Af Am	5	14
L771L (T2430C)	P	34 Af Am	159	8, 14
K820E	UV	9 Af Am, 1 Af	13	1, 14
P871L (C2731T)	P	1 Af Am	161	14, 20
P938P (A2933G)	P	2Af Am	0	8
E1038G	P	34 Af Am	203	8, 14
S1040N	P	2 Af Am	21	8, 14
S1140G (A3537G)	P	14 Af Am	19	2-4, 7, 8, 14
K1183R (A3667G)	P	36 Af Am, 1 Af	186	1, 4, 7, 8, 14
Q1200H	UV	2 Af Am	0	8, 14
L1260L (T4932C)	P	4 Af Am	0	8, 20
S1297F (C4009T)	UV	1 Af Am	0	4
G1371G (G4232A)	P	2 Af Am	0	8
T1561I	UV	1 Af Am	9	8, 14
L1564P	UV	2 Af Am	1	7, 14
L1605L (T4932C)	P	2 Af Am	0	8
Q1785H	UV	1 Af Am	0	7
E1794D	UV	1 Af Am	0	7
IVS1-10T/C	UV	2 Af Am	0	14
IVS8-58delT	UV	36 Af Am	99	8, 14
IVS12+12delGT	P	2 Af Am	0	8, 14
IVS16-?68G/A	P	34	95	8, 14
IVS18+? A/G	P	34	79	8, 14
IVS19+85delT	UV	1	0	14
IVS20+59ins12	P	2 Af Am	33	8, 14
IVS22+7 T/C	UV	1 Af Am	0	7
IVS22+8 T/A	UV	1 Af Am	0	7
IVS22+8 T/C	P	7 Af Am	8	8, 11, 14
IVS22+67 T/C	P	5 Af Am	0	7, 11
IVS22+78 C/A	UV	2 Af Am	0	7
IVS23-10C/A	UV	1 Af Am	6	14
C5817G (3'UTR)	P	18 Af Am	4	8, 14

TABLE 2. *BRCA2* Mutations in African American and African Breast Cancer Patients

BRCA2 mutations	Effect	#Af Ances Families	#non-Af Ances Families	Reference
Protein Truncating Mutations				
1536del4	FS	1 Af Am	0	2
1882 del T	FS	1 Af Am	2	14, 23
1991delATAA	FS	1 Af Am	0	22, 23
1993delAA	FS	1 Af Am	0	23
2001delTTAT	FS	1 Af Am	0	22
2816insA	FS	3 Af Am	5	2, 14, 22, 23
3034del4 (3036del4)	FS	1 Af	35	1, 14
4075delGT	FS	1 Af Am	16	14, 23
4088delA	FS	1 Af Am	1	14, 23
6696delTC	FS	1 Af Am	2	2, 14
Q2342X	N	1 Af Am	0	14
7436del4	FS	1 Af Am	0	15
7795delCT	FS	2 Af Am	0	2, 14
7907delTT	FS	1 Af Am	0	15
8643 delAT	FS	1 Af Am	0	23
9481insA	FS	2 Af Am	2	14
R3128X	N	1 Af Am	7	14
Splicing Mutations				
IVS13-2A/G	S	1 Af Am	0	15
Unclassified Variants and Polymorphisms				
P46S	UV	1 Af Am	0	14
P59A	UV	1 Af Am	0	14
N108H	P*	1 Af Am	1	2, 14
Q147H	UV	1 Af Am	0	14
A248T	UV	1 Af	1	1, 14
N289H	P*	1 Af	11	1, 14
Q713L	UV	1 Af Am	0	14
L929S	UV	1 Af Am, 2 Af	6	1, 14
S976I	UV	1 Af Am	3	14
N987I	UV	1 Af Am, 2 Af	6	1, 14
N991D	P*	1 Af	9	1, 14
S1172L	UV	2 Af Am	4	14
C1290Y	P*	1 Af Am	2	14
Q1396R	UV	1 Af Am	8	14
T1414M	P*	1 Af	4	1, 14
D1420Y	P	1 Af Am	113	14
D1781G	UV	1 Af Am	0	14
N1880K	P*	4 Af Am	4	14
N1880R	UV	1 Af	0	1
T1980I	UV	1 Af Am	0	14
H2074N	P*	2 Af Am	2	14
H2116R	UV	2 Af Am	7	14
K2339N	UV	4 Af Am	8	2, 14
Q2384K	UV	1 Af, 1 Af Am	7	1, 2, 14
H2440R	UV	6 Af Am	9	2, 14
A2466V	P*	8 Af Am	24	14
S2835P	UV	1 Af Am	2	14
I2944F	P*	9 Af Am	6	14
S3020C	UV	1 Af Am	0	14
M3118T	UV	1 Af Am	0	14
G3212R	UV	1 Af Am, 2 Af	0	1, 14
V3244I	UV	3 Af Am	8	14
T3357I	UV	1 Af Am	0	14
I3412V	P	14 Af Am	74	14
IVS6-19 C>T	UV	2 Af Am, 2Af	9	1, 14
IVS11+73 T>A	P*	1 Af	0	1
IVS18+109 G>A	UV	2 Af	0	1
IVS20-36 C>G	UV	1 Af	0	1
IVS24-T	UV	1 Af	0	1
IVS26+10T>G	UV	1 Af Am	0	14
IVS26+106 delT	P*	1 Af Am	0	1, 14

The Department of Defense
Breast Cancer Research Program Meeting

Era of Hope

PROCEEDINGS
Volume I

Presented at the
Hilton Atlanta and Towers
Atlanta, Georgia

June 8-11, 2000

BRCA2 MUTATIONS IN AFRICAN AMERICANS

Carolyn Whitfield-Broome, Yasmine Kanaan, Elikem Kpenu, Kim Utley, Georgia M. Dunston, Lawrence C. Brody

Howard University College of Medicine and Cancer Center, Dept. Biochem., Dept. Microbiol. Washington D.C.; National Human Genome Research Institute, Bethesda, MD

cbroome@fac.howard.edu

Specific mutations in the breast-ovarian cancer susceptibility gene *BRCA2* have been associated with different ethnic groups. The entire coding regions and flanking introns of *BRCA2* are being examined for germline mutations in African American breast cancer patients from 75 families at high-risk of hereditary breast cancer. To date about 75% of the gene has been screen for mutations by single stranded conformational polymorphism and the protein truncation test, and confirmed by DNA sequencing of variants. Six pathogenic, protein-truncating *BRCA2* mutations have been identified. Two frameshift deletion mutations, 4088delA or 8643delAT, were detected in female breast cancer patients diagnosed at an early age (<40 years) with no reported family history. Frameshift mutation 2001del4 was observed in a woman with early onset breast cancer from a family with one other case of breast cancer. A patient with ovarian cancer, from a family with multiple cases of breast and ovarian cancers, carried the frameshift mutation 4075delGT. Frameshift insertion mutation 2816insA was detected in a male breast cancer patient. Frameshift mutation, 1991del4, was observed in a male breast cancer patient from a family with one other case of breast cancer. A *BRCA2* missense variant, His2395Leu, of unknown functional significance was identified. Numerous polymorphisms and noncoding variants, which are probably not disease related, were also observed in the *BRCA2* gene. Determining the spectrum of *BRCA2* mutations in African Americans is important for genetic testing and genetic counseling of African Americans. A large number of different pathogenic *BRCA2* mutations are observed in the African American population; many of these protein-truncating mutations have not been reported in other populations. The numerous, distinct pathogenic mutations in *BRCA2* observed in African Americans reflect the high level of genetic variation in people of African ancestry. Because of this broad spectrum of distinct mutations, genetic testing for *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

This work was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8106 and in part by the National Race for the Cure, Komen Found.

450

Imbalance in wild-type hMSH2:hMLH1 protein ratio in lymphocytes identifies colorectal cancer (CRC) patients with hereditary nonpolyposis colon cancer (HNPCC) traits. *Z.P. Gao[1], Z.Q. Gao[1], S. Burkholder[1], T. Zhang[1], J.Z. Fields[2], A. Kairo[1], S. Ehrlich[1], B.M. Boman[1].* 1) Medical Genetics, thomas jefferson university, philadelphia, PA; 2) CA*TX Inc., Gladwyne, Philadelphia, PA 19035.

Identifying carriers of HNPCC traits can save lives and health care dollars. However, current genetics tests for HNPCC is costly and time consuming. Therefore, the need is to develop a more feasible assay to detect a germline HNPCC-causing mutation, particularly one that is sensitive, rapid, easy & inexpensive. Our approach to the problem of detecting HNPCC was to develop a quantitative immunoassay for wild-type DNA mismatch repair (MMR) protein levels (hMSH2 and hMLH1). Mutations in hMSH2 and hMLH1 account for the vast majority (>90%) of detectable germline mutations in HNPCC kindreds. And most (>70%) germline hMSH2 and hMLH1 mutations lead to a truncated protein product. We hypothesized that cells carrying a germline, truncation-causing, hMSH2 or hMLH1 mutation will have a 50% reduction in the corresponding full-length protein product. To test proof of principle for our assay, we used western blot analysis to estimate hMSH2 and hMLH1 protein levels in lymphoblastoid cell (WBC) lines from CRC patients. We tested 42 WBC lines established from CRC patients in our Familial Colorectal Cancer Registry and 8 WBC lines from healthy, unaffected individuals. Western blots were done using antibodies against the carboxyl end of both hMSH2 and hMLH1 proteins. All of the samples from healthy unaffected individuals had, on western blots, a) clearly identifiable bands for hMSH2 and for hMLH1 and b) nearly identical hMSH2/hMLH1 ratios. In 7 of the 42 WBC lines from CRC patients, we found decreased expression of hMSH2 or hMLH1 on western blot analysis and all 7 of these, subsequently, showed evidence of a mutation in corresponding MMR gene. Moreover, where DNA was available from fresh lymphocytes(5 of the 7), we found evidence for the mutation. Together these data demonstrate i) that our assay can be used to identify individuals with the HNPCC trait and ii) that this can be done practically and inexpensively.

451

Identification and characterisation of the familial cylindromatosis gene. *G.R. Bignell[1], W. Warren[2], S. Seal[2], M. Takahashi[2], E. Rapley[2], R. Barfoot[2], P. Biggs[2], A. Ashworth[2], M. Stratton[2].* 1) Team 78, The Sanger Centre, Hinxton, Cambridgeshire, UK; 2) Cancer Genetics Section, Institute of Cancer Research, Sutton, Surrey, UK.

We report the cloning of the gene for Familial Cylindromatosis (*CYLD*). Familial Cylindromatosis is an autosomal dominant genetic predisposition to multiple tumours of the skin appendages. The susceptibility gene has previously been localised to chromosome 16q12-q13 and has the genetic attributes of a tumour suppressor gene/recessive oncogene. The critical evidence for involvement of the *CYLD* gene in familial cylindromatosis is the identification of 21 germline mutations. We have also identified six somatic mutations, five from familial cylindromas and one from a sporadic cylindroma. All of the germline and somatic mutation are predicted to cause early protein termination. The *CYLD* gene has also been screened through a bank of other tumours, however, no mutations were detected from this set. Analysis of the protein sequence reveals three regions with homology to Cytoskeletal Associated Protein-Glycine conserved (CAP-GLY) domains, found in proteins that co-ordinate the attachment of organelles to microtubules, and homology to the catalytic domain of Ubiquitin Carboxy-terminal Hydrolase type 2.

452

Functional evidence for multiple tumor suppressor genes on the short arm of chromosome 8. *G. Chenevix-Trench[1], J. Arnold[1], P. Wilson[1], D. Trott[2], A. Cuthbert[2], R. Neubold[2].* 1) Queensland Inst Medical Res, Brisbane, Australia; 2) Brunel University, Uxbridge, UK.

The short arm of chromosome 8 frequently undergoes loss of heterozygosity in many different solid tumors, and homozygous deletions have been reported in prostate and squamous cell carcinomas. We have previously provided functional evidence of a tumor suppressor gene on this chromosome by the transfer of chromosome 8 into colorectal cancer cell lines by microcell fusion. Spontaneous deletions of the donor chromosome allowed us to map the location of the putative tumor suppressor region to a 5.2 Mb region at 8p22-23. We have now transfered chromosome 8 into ovarian (HEY) and breast (21MT and T47-D) cancer cell lines. HEY/8 hybrids containing all of the donor chromosome 8 grew normally in vitro but showed significantly reduced tumor formation in athymic mice compared to those that only contained the long arm of the donor chromosome. Analysis of hybrids containing only part of the donated 8p have allowed us to map two regions of suppression at 8p12-21 and 8p23. We have also obtained 17 21MT/8 and 5 T47-D/8 hybrids. All of these hybrids share in common the exclusion of several regions on the short arm of the donor chromosome. This suggests that they might contain tumor suppressor genes that convey a strong selective disadvantage in vitro. These data suggest that there are several tumor suppressor genes on the short arm of chromosome 8 and efforts are underway to narrow down their location prior to gene identification.

453

Towards identification of a senescence-associated gene / tumor suppressor gene (TSG) in the NRC-2 locus in human chromosome band 3p14. *B. Opalka[1], W. Bardenheuer[1], G. Marquitan[1], N. Werner[1], K. Juelicher[1], H. Topal[1], I. Horikawa[2], J.C. Barrett[2], J. Schuette[1].* 1) Innere Klinik (Tumorforschung), Universitaet (GH) Essen, Essen, NW, Germany; 2) NIEHS, Research Triangle Park.

Chromosomal alterations in human chromosome region 3p14 have been found in numerous tumor entities including lung cancer (LC) and renal cell carcinoma (RCC). Results of functional investigations as well as data concerning structural aberrations suggest the presence of at least one TSG in 3p14. The FHIT gene in 3p14.2 and the WNT5A gene in 3p14.3-21 have been shown to reveal TSG function in vitro and/or in animal models while mutations of these genes have been rarely detected in LC or RCC. We have previously established functional complementation assays using a YAC contig covering chromosomal band 3p14 and neighboring regions. Following retrofitting of YACs for the introduction of a mammalian selectable marker we established the YAC transfer by spheroplast fusion into a human RCC line showing a cytogenetically detectable deletion within 3p13-23. Using this approach we identified a 530 kb YAC clone within 3p14.2 which induced cellular senescence in vitro and reveals sustained suppression of tumorigenicity of transduced RCC cells in nude mice. This activity which maps differently from the FHIT gene and the WNT5A gene defines a novel TSG locus, NRC-2, in 3p14. Genomic sequencing was performed using PAC clones. Including data from the human genome project 60-80% of the entire YAC sequence are available now. Primers were synthesized corresponding to 6 ESTs and 15 predicted exon sequences as well as for 8 exon sequences identified in exon trapper experiments and are currently used to screen different cDNA libraries. These efforts should allow the isolation of the gene(s) responsible for the NRC-2 locus activity. Supported by Deutsche Forschungsgemeinschaft and foundation VerUm.

454

Two deletions in regions 6q22-23 and 6q25-27 are associated with immortalization of SV40 transformed cells. *J. Liu, A.K. Sandhu, N. Rane, R.S. Athwal.* Department Of Pathology,Fels institute, Temple University School of Med. Philedelphia, PA.

We have applied a 'Functional-Potional' approach to identify cell senescence genes on human chromosomes. Microcell mediated transfer of a gpt tagged intact human chromosome 6 or a part of the long arm(6q14-qter) restored senescence in human (Sandhu et al. 1994, PNAS 91: 5498-5502) and mouse SV40 immortalized fibroblasts. Segregation of the transferred chromosome 6, with the loss of gpt tag, led to the resumption of indefinite cell proliferation. While microcell hybrids were maintained in the MX selection medium, for the retention of the donor chromosome, immortal revertant clones arose among senescent cells. Reversion to immortal growth could result from the loss of the expression of the senescence gene due to a mutation or a deletion. Analysis of the revertant clones for the loss of the DNA markers, mapped in the region 6q22-qter, identified two deletions in the region 6q22-27. The results of this study revealed two independent deletions located at 6q22-23 and 6q25-27, suggesting the location of two senescence genes on the long arm of chromosome 6. In order to further define the position of the senescence gene(s), we have identified YAC and BAC clones corresponding to the deleted markers, by human genome data base search. A high resolution physical map of the region containing the senescence gene has been developed. Candidate BAC clones will be tested for the restoration of senescence by introduction into SV40 immortalized human and rodent cells.

455

BRCA2 mutations in African Americans. *Y.M. Kanaan[1], E. Kpenu[1], K. Utley[1], L.C. Brody[2], G.M. Dunston[1], C. Whitfield-Broome[1].* 1) Howard University College of Medicine, Departments Microbiol., Biochem.& Mol. Biol.; Cancer Center, Humam Genome Center, Washington, DC.20059; 2) National Human Genome Res. Inst., Bethesda MD 20892-4442.

Since the identification of the BRCA2 breast-ovarian cancer susceptibility gene, mutation analyses have been carried out in various populations revealing ethnic-specific mutations. In order to identify BRCA2 mutations in African Americans, seventy-five breast cancer patients from families at high risk of hereditary breast cancer were studied. The entire coding regions and flanking introns of BRCA2 have been screened for germline mutations by single stranded conformational polymorphism or the protein truncation test, followed by DNA sequencing. Eight protein truncating, pathogenic mutations have been detected. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described. Therefore, half of the pathogenic mutations observed are unique to African Americans. The other four pathogenic mutations (1882delT, 2816insA, 4075delGT, 4088delA) detected in African Americans have been previously reported in Caucasians. Two of the pathogenic mutations, 1991delATAA and 2816insA, were detected in male breast cancer patients, consistent with previous studies showing an association of BRCA2 with male breast cancer. One-half of the pathogenic mutations were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease. One rare missense variant of unknown functional significance was detected. Numerous polymorphisms and non-coding variants were observed. Considering our work and that of others, many different pathogenic mutations and many variants of unknown significance are observed in African Americans; therefore, BRCA2 genetic testing in high risk African American families needs to involve the entire coding and flanking sequences of the gene. This work was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8106 and in part by the Komen Found.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 8 Jan 2003

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the enclosed. Request the limited distribution statement for the enclosed be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Encl

ADB265840	ADB266633	ADB282069
ADB279138	ADB251763	ADB265386
ADB264578	ADB281601	ADB282057
ADB281679	ADB258874	ADB258251
ADB281645	ADB281773	ADB264541
ADB261128	ADB281660	ADB241630
ADB261339	ADB259064	ADB281924
ADB273096	ADB266141	ADB281663
ADB281681	ADB281664	ADB281659
ADB259637	ADB258830	
ADB256645	ADB266029	
ADB262441	ADB281668	
ADB281674	ADB259834	
ADB281771	ADB266075	
ADB281612	ADB281661	